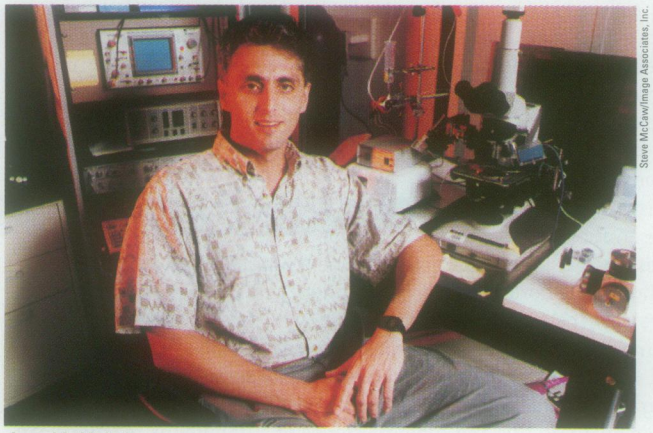
Getting Good Reception

The human brain contains hundreds of billions of neurons and connections, all busily engaged in transmitting the specialized electrical messages that allow the mind and body to sense and respond to the myriad stimuli encountered each moment of every day. In the NIEHS Laboratory of Signal Transduction, scientists study the mechanisms by which environmental agents disrupt these neural pathways, thereby altering neurophysiological processes and, sometimes, causing or exacerbating environmentally related disease. Neuroscientist Jerrel L. Yakel, who heads the Ion Channel Physiology Group within the Laboratory of Signal Transduction, studies neurotransmitter receptors, proteins found on the surface of nerve cells that bind endogenous and exogenous chemical signaling agents.

According to Yakel, neurotransmitter receptors, particularly those that are attached to ligand-gated ion channels, are often targeted by environmental toxicants such as animal toxins (including those produced by snakes, shellfish, and bees) and certain plant products (curare and nicotine, for instance), as well as by nerve gas agents. He says, "As these ligand-gated ion channels are also participating in neurological and cognitive development, interfering with them can have adverse health and cognitive effects such as you would see with lead or nicotine exposure *in utero* or in early childhood. The type of work I do tries to get at the fundamental issue of how neurons interact and communicate, with the aim of learning the cellular and molecular mechanisms whereby the natural and environmental compounds act."



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Jerrel L. Yakel

Yakel is particularly interested in identifying the molecular makeup and function of the nicotinic acetylcholine receptors (nAChRs) and serotonin 5-HT_3 receptors (5-HT_3Rs). The nAChRs and 5-HT_3Rs are ligand-gated ion channel receptors that facilitate the split-second communication between neurons that can trigger peripheral physiological responses (such as movement and the beating of the heart) and central responses (such as thought and sensory perception). Although the nAChRs and 5-HT_3Rs are expressed in many areas of the brain, it is still not known exactly how these receptors carry out their role in various brain functions.

Basic Neuroscience

Ligand-gated ion channels are pores in the membranes of cells that open and close in response to the chemical signals sent by neurotransmitters. Some of these channels can also be gated through the action of exogenous substances, such as nicotine in the case of the nAChR. The opening of these ion channels induces the influx of charged particles such as calcium ions (Ca^{2+}) into the cells, which, in turn, excites the nerve cells. This cascade of events allows the propagation of electrical messages throughout the brain and nervous system.

Once inside cells, Ca^{2+} regulates a variety of cellular functions through mechanisms that are still being studied and debated. Many of the processes regulated by Ca^{2+}, such as secretion, contraction, and cell division, are essential to cell function and survival. However, says Yakel, calcium can also be a bad thing; too much can cause cells to die—neuronal cell death associated with excessive Ca^{2+} elevation has been suggested as a possible contributing factor in several neuropathological conditions and neurodegenerative diseases, such as stroke, ischemia, and Alzheimer disease. The intracellular concentration of Ca^{2+} therefore must be tightly regulated.

Yakel believes that variability in the molecular makeup among different subtypes of the nAChR influences the efficacy of synaptic transmission in various regions of the brain, and may also be involved in the regulation of intracellular Ca^{2+} concentrations in cells. Subtle differences in the molecular makeup of each subtype determine its pharmacological and functional properties, which can vary widely depending on its location within the body and associated role in synaptic transmission.

Yakel's group studies the receptors found in the hippocampus, a region of the brain known to be important for learning and memory processing. There are nine known nicotinic receptor subunits that have been found in the hippocampus, six α subunits (α2–α7) and three β subunits (β2–β4). Nicotinic receptors assemble as pentamers, with five individual subunits grouped around a central pore. The nine subunits mix and match to form various receptor subtypes that function in different ways. Although there are hundreds of conceivable subunit combinations, Yakel believes only a few combinations occur naturally.

The nicotinic α7 subunit differs from the other nicotinic subunits in that it can form homomeric ion channels, meaning that five α7 subunits join to form a functional channel. (Channels usually include at least one α subunit and one β subunit.) In addition, α7 channels are highly Ca^{2+}-permeable, and they are believed to make up the majority of functional nicotinic receptors in certain parts of the brain. Yakel is particularly interested in determining the molecular makeup and functional properties of the other nicotinic receptors (which are referred to as the non-α7 nicotinic receptors) and the role played by these other subunits, about which relatively little is known.

The 5-HT_3R is relatively simple compared to the nicotinic receptor; only two 5-HT_3R subunits are presently known to exist. The 5-HT_3R channel, like the nicotinic receptor channel, is thought to be pentameric. Despite having only two subunits to draw from in forming receptor channels, 5-HT_3Rs are believed to be involved in several physiological responses such as cognition, pain perception, motor neuron activity, and sensory processing. Studies have shown that 5-HT_3R ligands can be used to effectively treat drug and alcohol addiction, schizophrenia, anxiety, cognitive dysfunction, and chemotherapy-related nausea.

Because nAChRs and 5-HT_3Rs as a whole are so similar in structure and function, and because they are expressed in similar locations in the nervous system, it is thought there may be some type of synergy between them. Yakel is investigating the possibility of interaction between the 5-HT_3Rs and the nAChRs, as well as possible overlap in function between the two. Acting on what he calls a "bit of a crazy idea"—the notion that different neurotransmitter receptor subtypes could coassemble into a heterogenous receptor—Yakel and colleagues have found that under certain circumstances, subunits of the two receptors combine into one functional receptor channel with unique physiological and pharmacological properties.

Combined Effort

In an article published in the 15 September 1998 issue of *Proceedings of the National Academy of Sciences*, Yakel and colleagues

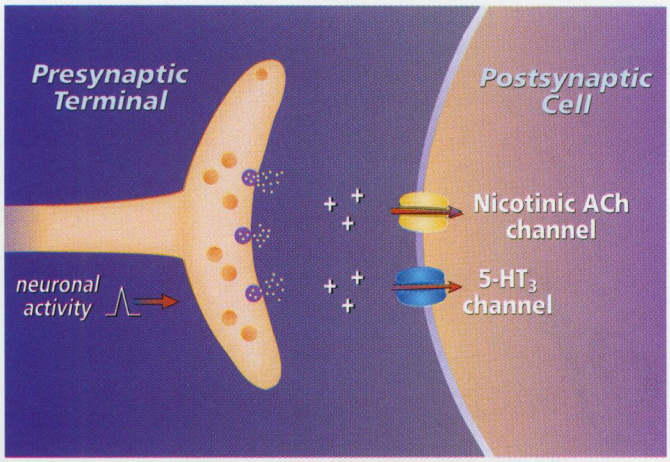
from the Netherlands and the United Kingdom first demonstrated that the 5-HT_3R subunit and the nAChR α4 subunit can coassemble into one ligand-gated ion channel that is activated by serotonin. The scientists found that, although the heteromeric assembly of the 5-HT_3 and nicotinic α4 receptor subunits and the homomeric 5-HT_3Rs had similar pharmacological profiles, they showed distinct sensitivities when it came to being blocked by a test antagonist. The demonstrated coassembly of subunits from different branches of the ligand-gated ion channel family may be an important clue in answering the question of how native neurotransmitter receptors can have such diverse physiological properties and functions.

To prompt this coassembly *in vitro*, Yakel and colleagues harvested oocytes from mature *Xenopus laevis* African frogs and injected them with cDNA encoding the 5-HT_3R subunit either alone or with one of six nicotinic receptor subunits. They measured the ion currents in these oocytes after 2–5 days to gauge the electrical activity of the cells. In examining the *Xenopus* oocytes, the scientists found that the coexpression of the nicotinic α4 subunit along with the 5-HT_3R subunit resulted in receptors that had distinctly different properties as compared to homomeric 5-HT_3Rs. Further analysis demonstrated that coexpression with the nicotinic α4 subunit significantly enhanced Ca^{2+} permeability over that of the homomeric 5-HT_3R channel. This increased permeability may be a mechanism used by the neurons to regulate synaptic transmission; increased Ca^{2+} influx into the presynaptic neuron could significantly influence the subsequent release of neurotransmitter.

The scientists also transfected human embryonic kidney (HEK) cells with cDNA encoding the 5-HT_3R either alone or with one of three nicotinic receptor subunits. They wanted to test whether the nicotinic α4 and 5-HT_3R subunits would co-immunoprecipitate—that is, whether both protein subunits would co-localize during an immunoblot assay—as might be expected if the subunits actually coassembled to form a functional channel. They found that the two subunits did indeed co-immunoprecipitate. The team also confirmed in HEK cells what they had found in the *Xenopus* oocytes, that the opening of the 5-HT_3/α4 channels resulted in enhanced Ca^{2+} entry. Because Ca^{2+} plays such an important role in the cell signaling process, this finding may be particularly important.

Through the Receptor

In order to further investigate how the nicotinic α4 and 5-HT_3R subunits interact, Yakel continued the line of investigation in a study that was published in the 12 February 1999 issue of the *Journal of Biological Chemistry*. In the study, Yakel and postdoctoral investigators Steve Kriegler and Sterling Sudweeks sought to determine whether the nicotinic α4 subunit formed part of the pentameric pore along with the 5-HT_3R subunit. The team was able to confirm that, in fact, the two subunits combined to form the pore of the ion channel.



Cascade of events. Intercellular communication in the nervous system occurs at specialized sites known as synapses. Neurotransmitters, such as serotonin and acetylcholine, are packaged in membrane-encased vesicles in the presynaptic terminal (left) and are released in response to neuronal activity. They cross the synaptic cleft and bind to and activate various ligand-gated ion channel receptors on the postsynaptic membrane (right), allowing the electrical activity to pass from cell to cell throughout the brain and nervous system.

To demonstrate this, the scientists inserted, through site-directed mutagenesis, the amino acid cysteine (which contains a reactive sulfhydryl group) into the nicotinic α4 subunit. The cysteine was positioned at a site that would be expected to line the pore of the channel if the nicotinic subunit did indeed contribute to the lining of the pore. The team coexpressed the mutant nicotinic α4 subunit along with the 5-HT_3R in *Xenopus* oocytes and then searched for the presence of this cysteine residue in the pore of the channels by using a molecular scanning technique known as the substituted-cysteine accessibility method.

When the team coexpressed the mutant nicotinic α4 subunit along with the 5-HT_3R subunit, they found that serotonin-activated responses were significantly blocked by a methanesulfonate compound that reacted with cysteine residues when the mutant nicotinic subunit was present. This, Yakel says, is further proof of coassembly between these two subunits. Furthermore, it demonstrates that both subunits actually contribute to the lining of the pore of the 5-HT_3/α4 channel. According to the report, the addition of a nicotinic subunit to the pore of the 5-HT_3R may be a general strategy used in neurons to increase the possible number of 5-HT_3R subtypes and therefore produce the wide range of divergent properties reported for native 5-HT_3Rs.

Possible Applications

Coassembly of the 5-HT_3R subunit and the nicotinic α4 subunit into a novel receptor might help to explain the diversity of the functional and pharmacological properties noted among native 5-HT_3Rs, a diversity that would seem unlikely, if not impossible, given the known 5-HT_3R variants that have been cloned thus far. Yakel's discovery raises intriguing pharmacological possibilities for the further study of nAChR and 5-HT_3R combinations.

Some acetylcholinesterase inhibitors, such as tacrine, are being used today to reduce some of the cognitive deficits associated with Alzheimer disease. Nicotinic receptor ligands are also being developed to aid in cognition, addiction, and analgesia. However, these agents often suffer from a lack of specificity; they target nicotinic receptors throughout the entire nervous system rather than attacking receptor subtypes responsible for specific responses, and they may cause severe cholinergic side effects including hypertension, neuromuscular paralysis, and seizures.

Yakel says that understanding the molecular makeup and functional regulation of the nicotinic and 5-HT_3 receptors in the different regions of the nervous system is critical for developing new pharmaceutical therapies. "It is clear that the work from my lab is very basic in orientation," he says. "Nevertheless, our work will help to understand the molecular targets at which endogenous neurotransmitters act to control synaptic communication within the brain. As scientists are discovering daily more and more about the molecular details involved in disease (e.g., genetic and environmental links to disease), it is critical to understand what the individual protein molecules are doing at the cellular level if we are to try to understand the nature of these diseases."